Newsletter for August 2024

Integument review project update - Scales chapter is up!

By Zennith | August 28, 2024

We are excited to announce that the next chapter in our Integument Review project is finished and uploaded for anyone to read!

Scales are the focus of this chapter, especially those in reptiles. We briefly mention scales in other species as well, like pangolins and fish.

The skin is one of the most distinctive features of any species (and therefore crucial for species affirmation). Yet, the skin is frequently subjected to large forces or challenges, is constantly self-renewing itself to repair damage, is richly innervated by various types of sensory cells, and frequently performs actions (such as hair piloerection or sweating). The skin is very much *not* a passive organ. Our Integument Review project aims to understand the skin as a prerequisite for engineering.

We expect that in many cases, engineering performed on the skin will be primarily bioengineering. Whether that means gene or cell therapies, or bioprinting, these systems should probably be living and self-regulating once established in the patient.

Earlier this year, we posted chapters representing the basic properties of fur and of human skin, respectively.

It might come as little surprise, but scales are pretty different from something fuzzy! I have to admit, I assumed scales wouldn't really be that different from non-scaly skin...but they are! There's a lot going on.

In human skin, the epidermis is largely composed of mostly-uniform keratinocytes that express the same set of structural molecules (e.g. keratins), with newer cells building epidermis from the bottom to replace older, dead keratinocytes as they are lost.

There's a lot more going on with reptiles. This is nicely illustrated with what tissue layers look like for lizards and snakes (squamates). Different layers within the scale epidermis are anatomically distinct, have different material properties, and are made from different structural molecules.

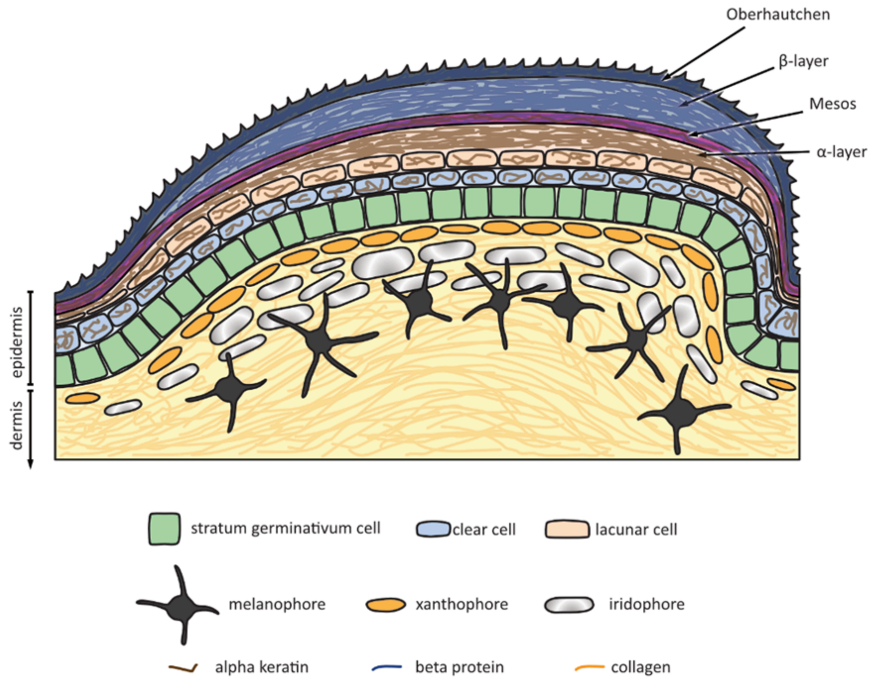


Figure 1: Tissue layers in squamates. *Scale plates have thick, strong beta and alpha layers, while hinges between scales are thinner and more flexible. The stem cells that renew scale layers are shown in green. Cells that affect scale color - melanophores, xanthophores, and iridophores - are also shown. Additional description starts on page 3.*

In order to accomplish scaly hides, as with the other integument types, we need 3 things:

- Descriptions of the system's composition and apparent properties
- Mechanistic understanding of the systems, including genes, signaling molecules, and other cellular and molecular details.
- Engineering steps to implement relevant portions of the natural systems in a different context (i.e., patients).

This chapter is primarily focused on the system's composition and apparent properties, but has several key molecular details and some engineering-related commentary as well.

The information tends to fall into one of 4 topics: (1) types of scales; (2) epidermal layering and how layers are replaced; (3) material properties including stretchiness; and (4) coloration and pigmentation. Those topics approximately map to sections 3.1; 3.2-3.3; 3.4-3.5; and 3.6-3.7, respectively.

Types of scales

Snake scales are probably the first scales many folks think of. The scales on snakes overlap from one row to the next, helping make for a skin covering that is flexible (at least, flexible along the axes that matter to snakes), yet serves as durable armor and shielding from the elements.

Snake scales are an example of *overlapping scales* (yep, that's their scientific name, nothing latin here). The degree of overlap can vary by species, and some "overlapping" scales, such as in some areas of the skin in iguanas, have nearly zero overlap at all. Some examples of how overlap can vary are given in Figure 2.

Overlapping scales are one of the types of scales that squamates can have. Squamates can also have *tuberculate scales*, which are more rounded in appearance, and don't overlap each other.

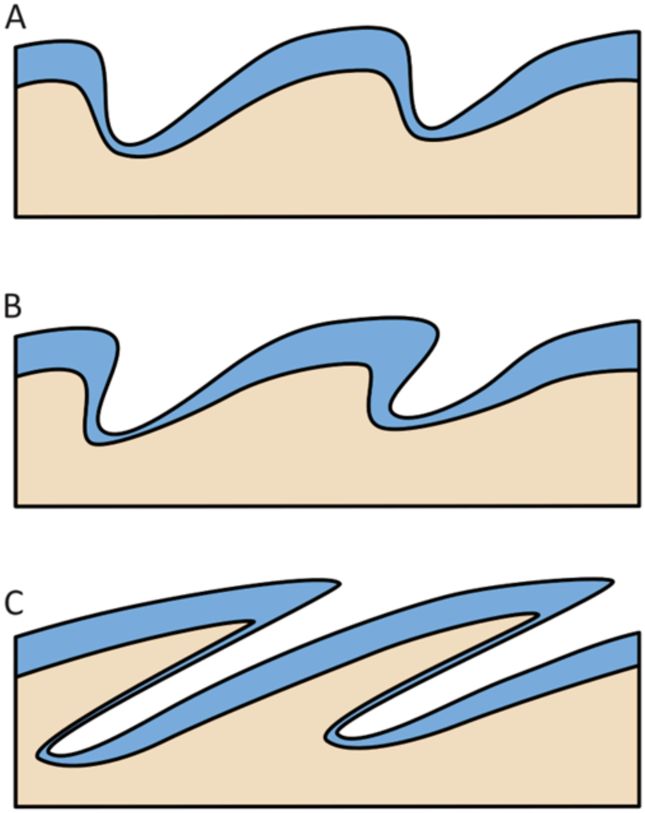


Figure 2: Examples of squamate overlapping scales. *A: Iguana tail scales have essentially no actual overlap. B: Iguana trunk scales have modest overlap. C: Snake scales have extensive overlap.*

The scales on crocodiles and alligators are pretty different from the above. Called scutes, these scales are broader, and frequently have underlying reinforcement from bony structures in the dermis.

There's more!

Did you know that birds are reptiles? (this is getting to be pop science now, but plenty of people haven't heard yet!)

Okay. Did you know that birds have scales? Scales cover the legs and feet of chickens, for example; it's not just skin.

And then… did you know that the scales on birds *probably evolved from feathers*? The scute-shaped scales that cover bird legs are not evolutionarily equivalent to the scales of other reptiles like crocodiles (Wu et al, 2018).

That fact blew my mind, personally!

Another type of scale that is worth mentioning in this little preview is the type that pangolins have. Pangolin scales are large, relative to body size, as compared to other scale types we have been looking at. Pangolin scales overlap each other, visually reminiscent in shape to enlarged snake scales. However, distinct from reptile scales, pangolin scales are extruded outwards from the skin's surface, with the tip of each scale being oldest, and the base of each scale being the youngest.

There are molecular and cellular similarities between pangolin scales and how fingernails grow in primates, and that means there is a good chance that pangolin scales in patients might be one of the easier kinds of scales to implement.

Still, it is important to keep in mind that pangolin scales look different from most other scale types we've been looking at, and there will be less ability to customize the color of them than reptile-like scales. While we know some patients will enthusiastically choose pangolin-like scales for identity affirmation, others will find them unsuitable, preferring reptile-like scales like overlapping, tuberculate, and/or scutate scales. Further research into both pangolin-like and reptile-like scales is of course needed.

Tissue layers and epidermal replacement

It took a surprising amount of effort to dig into information about reptilian scales, until we got to a point where we felt like we understood what they're composed of and how they work.

Much of the existing scientific literature on PubMed for reptile scales is…decent, but it is rare to find good review articles, and even more rare to find good illustrations of the concepts. We owe a lot to two giants in the field: P. F. A. Maderson, and Lorenzo Alibardi.

Maderson published some key papers in the 1960s (1965, 1966) with diagrams that are more clear and comprehensive than almost anything else we've encountered since (yes, seriously!). They stand up to time, and have only needed slight updating to account for (relatively) more recently-identified features of scales, such as a "mesos layer" in lizards and snakes, and better understanding of molecular details.

Meanwhile, Alibardi-authored publications represent something like, literally a third of our citations for scales. He is *that* prolific of a reptile researcher!

So, for the tissue layers of scales: we learned that the anatomy of the tissue layers, and how epidermis is replaced/renewed over time, is very different depending on the type of reptile you are looking at.

The 260 million years or so of evolution that separates lizards and snakes from crocodiles, turtles, and birds is apparently not a joke!

There's several clades of reptiles that currently exist. Turtles, birds, and crocodiles are *archelosaurs*, whereas lizards, snakes, and tuatara (a lizard-shaped non-lizard in New Zealand) are *lepidosaurs*. We selected two groups to focus on when writing about tissue layers and epidermal replacement processes: crocodilians (crocodiles and alligators) and squamates (lizards and snakes). Those groups represent the concepts we need to pay attention to when researching how to implement reptilian scales in patients.

For lizards and snakes, their scale layers are arranged as is drawn in Figure 1. Starting from the top, there's an Oberhautchen and a beta layer (both of which are pretty strong, hard layers), followed by a mesos layer (not quite as hard), then an alpha layer (tough, but able to bend), then a few populations of cells. Several of these layers are thinner in the flexible hinge regions between scales.

Stem cells under those other cells - the stratum germinativum, or basal layer - give rise to all the cells that generate new epidermal layers. As the cells divide and differentiate, they push everything above them upwards. Eventually, a full new set of inner epidermal layers has been created, underneath the outer set of layers.

Notably, clear cells are kinda like…explosive bolts used on rockets, when spent boosters are separated from subsequent rocket stages. They're secure when you want them to be, but can be controllably removed when appropriate. When clear cells are removed, the older, outer epidermal layers above them can fall off; ablation of the clear cells makes the scale replacement/renewal cycle possible.

Meanwhile, crocodiles and alligators are known to have a more gradual scale replacement/renewal process that does not involve shedding. Since they are not stuck waiting around for their scales to shed, this is why crocodiles are able to see you in a while, and why alligators can see you later.

New cells are still produced from a basal cell layer at the bottom of the epidermis, and those new cells push older ones upwards. However, unlike the distinct layering in squamates (Ober, beta, mesos, alpha…), crocodilians have a simpler arrangement. The plate-like regions of scales are made from cells that make a stiff structural protein, whereas the hinge regions between scales are made from other cells that make a more flexible (though still tough) structural protein.

Material properties of scales
What would it feel like if you poked a viper?

A komodo dragon?

A crocodile?

What if you tugged on one of their scales, would it deform any? How about the flex between their scales, how bendy are they? I'm sure those creatures wouldn't mind. I'm confident that a viper, komodo dragon, and crocodile would absolutely not bite you if you tried poking them. I'll wait right here for you to try it out for yourself. :3

The specific mechanics and material properties are important to get right. Any scaly covering being considered for a patient has to be sufficiently flexible *in the relevant axes of movement*, while being sufficiently strong and tough, including on the areas facing the greatest wear or stress.

The shape and arrangement of scales - and the hinges between them - need to be adapted to movements, especially at the shoulders and hips. Voronoi patterns, modeling scales as polygons and hinges as the edges between polygons, will be a helpful basis for modeling the optimal shapes and arrangements of scales, as well as the ability of the scale hinges to bend and stretch in different directions (anisotropy).

If additional flexibility in a region is needed, the hardness of scale plates could be reduced, or the width of the more flexible hinges could be increased.

As well, we briefly mentioned this above, but different parts of scaly skin are built from different structural molecules. The main, hard part of the scales of lizards and snakes has a relatively thick layer of keratin-associated beta protein, with a reinforcing layer of alpha keratin under it. The flexible hinge area between the scales has essentially no beta protein, and a thinner layer of alpha keratin.

Crocodilians use a similar strategy - the hard parts have thick layers of beta protein, whereas the hinges have thinner and more flexible layers of alpha keratin.

Regardless of the type of scale, the balance of alpha keratins and beta proteins will be important to get right.

Other structural contributors are also present. The dermis contains a lot of collagen and elastin, contributing stretchiness, flexibility, and the ability to spread out forces encountered on the skin over a wider area. In some animals, the dermis also has bony plates that further reinforce the scales of the epidermis.

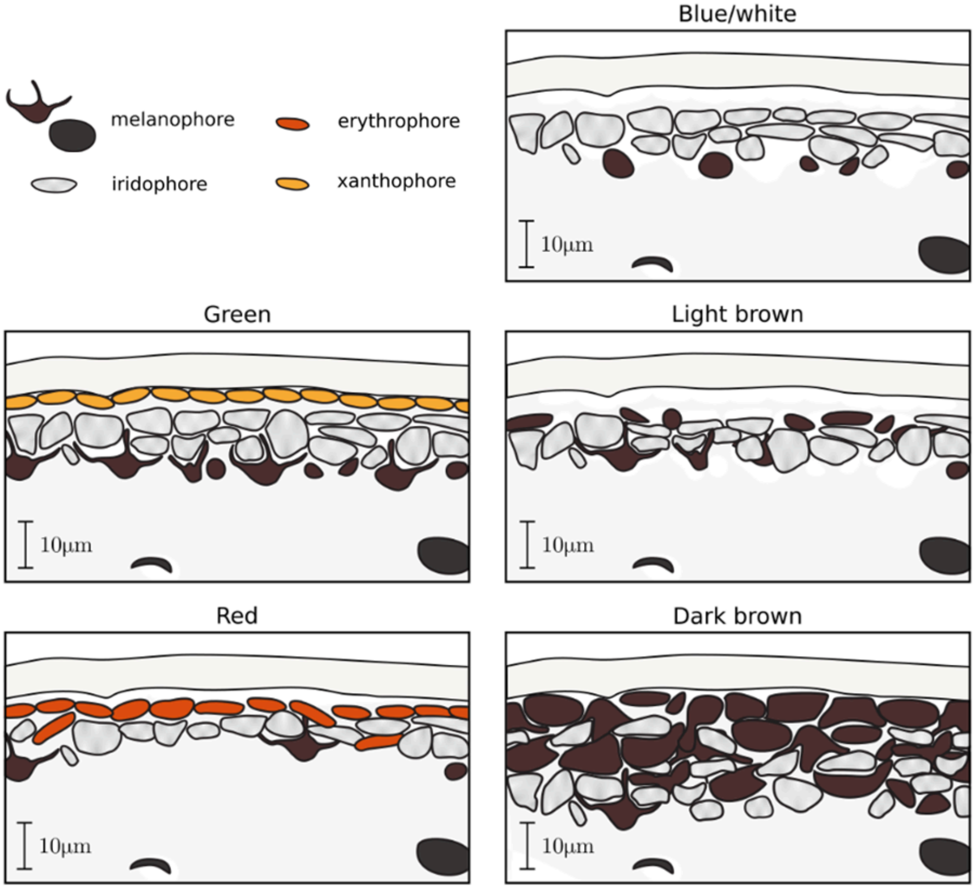
While there are currently few research papers that have explored the impact of material composition on scale properties, aspects like Shore hardness and Young's modulus are routinely measured in other fields of research and could be applied here. It would be interesting to measure these and other parameters for existing species. In the future, performing measurements like that would be very helpful in the course of optimizing tissue and genetic engineering approaches when we are making new scales in the lab.

Pigmentation and scale appearance
Reptile coloration is highly varied and can involve some vibrant colors. This is accomplished with a surprisingly limited palette: mainly, melanophores or melanocytes (both of which can either be black or brown), xanthophores (yellow cells), erythrophores (red cells), and iridophores (shiny cells).

In geckos, these cells are arranged in specific patterns in the dermis (Figure 3):

- Green scales are accomplished by layering xanthophores on top of iridophores and melanophores.
- Red scales have erythrophores on top of iridophores and melanophores
- Blue or white scales have just iridophores and a very small number of melanophores
- Brown scales have a lot of melanophores along with some iridophores.

Figure 3: Relationship between scale color and pigmentation/color-related cells used to achieve the effect. *The interactions between cells can result in new apparent colors – such as how xanthophores (yellow), iridophores (shiny), and melanophores (brown or black) result in a green effect.*



In crocodilians, pigment cells are also (mostly) in the dermis, even if the naturally occurring colors in these species tend to be more muted. A lot of crocodilian coloration comes from melanocytes and melanophores. Melanocytes in crocodilians actually *inject* melanin pigment into the cornifying epidermis, so that as the keratinocytes die, melanin gets incorporated into the non-living scale material.

Meanwhile, melanophores allow crocodilians to change their color according to the environment (Merchant et al, 2018). Their melanophores have a store of melanin inside them, which is moved around as needed. For better camouflage in dark environments, the melanophores distribute the melanin out to the tips of the cells, spreading it out, absorbing more light passing through the skin. Whereas, in bright environments, melanophores retract their melanin, making the cells less dark, allowing more light to pass and reflect.

We identified additional factors influencing scale appearance as well. They include subsurface scattering, reflectivity, the impact of surface roughness, and even the amount of beveling of scale edges. These and other details are included in the chapter itself.

Scaling to new heights

We learned a lot through writing this chapter - scales are, indeed, not fur!

To be less flippant, there is no obvious way to alter, for example, a hair growth program, with different gene expression levels, to create a scale. As well, there are differences in the types of cells and structural molecules (it's not just "add more keratin"), and even in the layout and types of pigment-containing cells.

While some things are common between reptile and primate skin, like the upwards migration of keratinocytes as they become nonliving structural material, several of the differences are likely to require genetic engineering and cellular therapies.

This chapter has provided us with a description of what scales are made of, how they grow, relevant aspects of their material properties, and pigmentation and appearance. We've got the system's composition and properties locked in.

Our next step for scales will be reviewing molecular and cellular specifics in greater detail - like more specific description of differentiation pathways, transcription factors, morphogens and other signaling molecules, and digging into more specifics of the structural molecules. It is likely that there will be several remaining unknowns after this literature research, because the cellular and molecular mechanisms in reptiles are not as well known as they are in mammals. This is fine too - we will figure out the remaining unknowns that our organization has to fill in with exploratory or hypothesis-driven science, which would involve histology and/or RNA sequencing (which our personnel have experience with).

Finally, we'll be able to plan out options for engineering and implementing scales, as well as ways we see of subdividing problems, methodically solving them, and optimizing parameters as needed.

Outlook for the project

We have already completed descriptive chapters for fur/hairs and for human skin. The last remaining descriptive chapter in front of us is for feathers.

The Properties of Feathers chapter still needs some more work, and the current plan is that it will not include the embryonic development of feathers that we mentioned in our April 2024 newsletter. Feather embryogenesis is slated for Chapter 7, since that chapter is where the bulk of cellular and molecular details are being discussed.

The Integument Review project has been a massive team effort so far, and will continue to be so! It requires different skillsets - physics, biochemistry, molecular cell biology, embryogenesis, and resourcefulness in finding and integrating different sources of information. Tiltwolf, Lathreas, Tiger, and myself have done the most work recently on scales, but we are also thankful for the contributions of former team member Rakeela who also helped with this chapter. Please join me in thanking them!

As always, thank you to everyone who is helping support our work - whether you're helping bring in new, exciting ideas in our community, or supporting us financially, or anything else, we appreciate it! Many paws make for a lighter load. :3

The Properties of Scales chapter can be found at https://freedomofform.org/research/project-integument-review/